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Shape effects of electrospun fiber rods on the tissue distribution and antitumor efficacy

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ABSTRACT

The significant impact of drug-loaded nanocarriers on cancer chemotherapy lies in the ability to specifically target to tumors with alleviated systemic toxicities. In the current study, a versatile and scalable method has been developed to construct fiber rods from electrospun fibers by ultrasonication using encapsulated NaCl nanoparticles as void-precursors. The shape effects of doxorubicin (DOX)-loaded fiber rods with an average diameter of around 500 nm and different lengths are determined on the blood circulation, tumor accumulation and cellular uptake. Compared with microspheres, fiber rods indicate an up to 4-fold higher accumulation in tumors and an up to 3-fold longer terminal half-life of plasma DOX levels after intravenous injection. Fiber rods with shorter lengths show a significantly higher in vitro cytotoxicity to tumor cells, a higher DOX accumulation and cell necrosis in tumors, and a significantly lower metastasis in lungs. Among fiber rods with different lengths, fiber rods with an average length of 2 μm induce significantly higher inhibition on tumor cell proliferation and induction of cell apoptosis, as wells as no detectable metastatic nodules in lung sections. Therefore, the shape effects of electrospun fiber rods hold great potential for enhancing systemic circulation and directing biodistribution to improve therapeutic outcomes.

1. Introduction

Chemotherapy represents the most widely used method for cancer treatment, employed alone or in combination with surgical removal or radiotherapy. While significant advances have been made in the conventional systemic chemotherapy through directly killing tumor cells, it still suffers from many side effects such as immunosuppression, nausea and hair loss. This is due to the non-specific distribution of chemotherapeutic agents in healthy tissues, and only a small percentage of administered drugs accumulate in tumors [1]. Invariably, the side effects impose a dose reduction, treatment delay, or discontinuance of therapy, thus substantial improvements need to be made in the target therapy to reach the next level of clinical relevance. In addition to the development of novel anticancer drugs, various drug delivery systems such as liposomes, nanoparticles and micelles, have been developed to improve the site specificity and bioavailability of drugs, thereby enhancing the therapeutic efficacy and minimizing the systemic toxicity [2].

To achieve a targeting capability of drugs and their formulations, there are several challenges needed to be addressed, including sufficient residence in the circulation before arriving at tumor sites, efficient travel within tumors and uptake into tumor cells, as well as a prompt release at the intended site for realization of therapeutic functions. One of the strategies is the attachment of targeting ligands with drugs or at the surface of nanocarriers, which bind to appropriate receptors overexpressed by tumor cells [3]. Alternatively, the passive targeting strategy takes advantages of the distinctive pathophysiological features of tumors and allows a preferential accumulation in tumor areas with leaky vasculatures, commonly referred to the enhanced permeation and retention (EPR) effect [4]. Though the first example of targeted liposomes was described in 1980s, these targeting technologies have not made a significant clinical impact on human health. Loomis et al. indicated that the in vivo tumor accumulation of drug and survival time of animals were not different between folate receptor-targeting and non-targeting liposomal doxorubicin (DOX), despite promising results from in vitro uptake studies demonstrating that the inclusion of folate increased the cellular uptake [5]. Some targeted drug delivery systems have already shown clinical efficacy, but there are contradicting data in the active targeting strategies regarding the added benefits for the inclusion of targeting molecules. Most likely, the recognition of targeting ligands by the reticuloendothelial system (RES) accelerates the clearance process, which offsets the benefit of active drug targeting to tumors [6]. Thus, an efficient passive targeting is also beneficial to the nanocarriers based on ligand-mediated active targeting, but it is known that over 95% of administered doses are accumulated in organs other than tumors, particularly in liver, spleen, and lungs [7]. The passive targeting is largely mediated by the physicochemical properties of nanocarriers, and

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attempts have been made to engineer drug carriers exhibiting a prolonged half-life in the bloodstream and a lower uptake by RES and non-target tissues [8].

The shapes of nanocarriers have shown significant effects on the circulation half-life in blood and the distribution in tumor tissues. Geng et al. tested the effects of nanoparticle shapes on the circulation time following a systemic injection of 3.5 μm-long filomicelles and spherical nanoparticles of 200 nm. The filomicelles circulated for up to one week in rodents, whereas the spherical nanoparticles were cleared within 2 days [9]. Thompson et al. indicated that rod-shaped microparticles (1 or 2 μm equivalent spherical diameters) with high aspect ratios (length-to-diameter) displayed significantly improved margination compared to microspheres. But nanorods (500 nm equivalent spherical diameters), even with a high aspect ratio, did not exhibit enhanced margination compared to that of equivalent nanospheres under the high shear rate and disturbed blood flow [10]. Decuzzi et al. investigated the biodistribution of silica microspheres with diameters ranging from 700 nm to 3 μm and non-spherical hemispheres, cylinders and discoids after intravenous injection into tumor-bearing mice. It was indicated that larger particles of non-spherical shapes may provide concentrations at intermediate sites that afford the deployment of novel modes of anticancer therapy, although smaller particles may well provide larger initial concentrations at tumor sites via different mechanisms of extravasation [11]. Therefore, an intensive consideration is needed in the evaluation of the contribution of particle sizes and shapes towards blood circulation, tissue distribution and cellular internalization [12].

With recent advances in the "bottom-up" and "top-down" approaches, the development of well-defined polymeric nanostructures of different shapes has become possible. The bottom-up approach relies on a spontaneous self-assembly of amphiphilic block copolymers to form elongated micelles as a result of the thermodynamic incompatibility between different blocks. This chemistry-centered approach is relatively simple and cost-efficient, particularly for nanoparticles of sub-100 nm range, but is limited to polymers with specific components and structures [13]. In the "top-down" strategy, the preparation of non-spherical polymeric nanoparticles mainly includes particle replication in nonwetting template (PRINT) and membrane stretching methods. The PRINT method is a high-resolution molding technology which allows for the formation of nanoparticles in the micro- and nano-scale size ranges with an aspect ratio as high as 60 [14]. Non-spherical shape is also fabricated by stretching spherical particles in one or two dimensions after embedment into poly(vinyl alcohol) (PVA) films, and rods or elliptical disks can be obtained with aspect ratios ranging from 2 to 15 [15]. The top-down approaches indicate advantages in the fabrication of nanostructures with a wide range of shapes from sub-micron to micron at will, but shows limitations in the scale-up production and protection of entrapped drugs.

Electrospinning is a versatile technique for nanofiber fabrication and possesses such advantages as simplicity, cost-effectiveness, flexibility and potential to scale up. Electrospinning provides an opportunity for direct encapsulation of drugs into a broad range of polymers with a high loading efficiency [16]. Electrospun fibers have been implanted intratumorally or adjacent to tumor tissues for those unresectable or inoperable solid tumors, or at the resection margins after surgical removal of solid tumors [17]. But the physical implants require direct accessibility through surgical procedures, having a large invasiveness. In this context, fiber rods were constructed from electrospun fibers under ultrasonication, and the lengths were modulated by the amount of NaCl nanoparticles encapsulated into fibers. In order to determine the cellular uptake and tissue distributions of fiber rods, coumarin 6 was loaded into poly(styrene-maleic anhydride) copolymers (PSMA) to obtain coumarin-containing fiber rods (RD_{COU}). PSMA fiber rods were selected because they are not biodegradable, enabling evaluation of the temporal biodistribution without resorption as a confounding variable. The cellular uptake of fiber rods with different lengths was evaluated on tumor cells and macrophages, and the distribution in tumors and other tissues was determined after intravenous injection. Poly(ethylene glycol)-polylactide (PELA) was used to construct DOX-loaded fiber rods (RD_{DOX}) of different lengths. In comparison with microspheres, the cytotoxicity, pharmacokinetics, antitumor efficacy and antimetastasis capabilities of RD_{DOX} were evaluated on tumor-bearing mice.

2. Materials and methods

2.1. Materials

PELA (Mw = 42.3 kDa, Mw/Mn = 1.23) containing 10 wt% of PEG was prepared by bulk ring-opening polymerization using stannous chloride as catalyst [18]. PSMA (Mw = 170 kDa, Mw/Mn = 2.5) containing 14.8 mol% of maleic anhydride was obtained from Shanghai Zhaocheng Science and Technology Development Co., Ltd. (Shanghai, China). Coumarin 6, 4′,6-diamidino-2-phenylindole (DAPI), trypsin and dialysis bags (1 kDa cutoff) were procured from Sigma-Aldrich Inc. (St. Louis, MO). Sodium bis(2-ethylhexyl) sulfosuccinate (AOT, 96%) was purchased from Aladdin Industrial Co., Ltd. (Shanghai, China), and DOX hydrochloride was from Dalian Mellon Biological Technology Co., Ltd. (Dalian, China). Rabbit antimouse antibodies of caspase-3 and Ki-67, goat antirabbit IgG–horseradish peroxidase (HRP) and 3,3-diaminobenzidine (DAB) developer were obtained from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). All other chemicals and solvents were of reagent grade or better, and received from Changzheng Regents Co. (Chengdu, China), unless otherwise indicated.

2.2. Preparation of drug-loaded fiber rods

Electrospun fiber rods were constructed by scission of electrospun fibers containing NaCl nanoparticles by ultrasonication. NaCl nanoparticles with an average size of around 500 nm were prepared using reverse microemulsions as described previously with some modifications [19]. Briefly, 10 mL of $CaCl_2$ solution in formamide (4.4%, w/v) was added dropwise into 10 mL of AOT solution in n-heptane (16%, w/v) to form microemulsions. After kept stirring for 1 h at room temperature, 15 mL of acetone was added for emulsion breaking, followed by ultrasonication for 5 min to disperse the formed nanoparticles. The suspensions were centrifuged to collect nanoparticles, followed by acetone washing and vacuum dried. Drug-loaded electrospun fibers with inoculated NaCl nanoparticles were prepared as described previously with some modifications [20]. Briefly, 0.5 g of polymers (PSMA or PELA) were dissolved in dry dimethyl formamide, and different amount of NaCl nanoparticles from 25 to 125 mg were added into polymer solutions. The formed suspensions were placed into a 1-mL syringe and pushed by a microinject pump (Zhejiang University Medical Instrument Co., Hangzhou, China) at a flow rate of 0.4 mL/h. The electrospinning was performed under 20 kV/10 cm using a high-voltage power supply (Tianjing High Voltage Power Supply Co., Tianjing, China). Fibers were collected on an aluminum foil wrapped on a grounded rotating mandrel. After removal of solvent residues under vacuum, the fibrous mats were put in a glass vial containing 10 mL of distilled water, which was cooled by a water-ice slurry to maintain the processing temperatures below 25 °C. Ultrasonication was carried out using a Vibracell 500 W sonicator (Techcomp Limited Co., Beijing, China) with a probe diameter of 13 mm, working at 20 kHz. The total run time was 3 min under an amplitude of 60% with a 2 s ON and 2 s OFF.

DOX hydrochloride was mixed with excess triethylamine in dimethyl sulfoxide overnight to obtain a DOX base, which was added into above PELA solutions to prepare RD_{DOX}. For comparison, DOX-loaded PELA microspheres (MS_{DOX}) and coumarin-loaded PSMA microspheres (MS_{COU}) with a diameter close to that of fibers were prepared by the solvent evaporation process as described previously with some modifications [18]. Briefly, polymer (PSMA or PELA) and drugs (DOX or coumarin 6) were dissolved in methylene chloride. The solutions were added dropwise into distilled water containing 5% of PVA, followed by